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J. Nutr., July 1, 2005; 135 (7): 1809S-1812S.
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Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion
D. Paddon-Jones, M. Sheffield-Moore, A. Aarsland, R. R. Wolfe and A. A. Ferrando
Am J Physiol Endocrinol Metab, April 1, 2005; 288 (4): E761-E767.
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Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids

Douglas Paddon-Jones, Melinda Sheffield-Moore, Daniel L. Creson, Arthur P. Sanford, Steven E. Wolf, Robert R. Wolfe, and Arny A. Ferrando
Department of Surgery, University of Texas Medical Branch and
Metabolism Unit, Shriners Burns Hospital, Galveston, Texas 77550

Paddon-Jones, Douglas, Melinda Sheffield-Moore, Daniel L. Creson, Arthur P. Sanford, Steven E. Wolf, Robert R. Wolfe, and Arny A. Ferrando. Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. *Am J Physiol Endocrinol Metab* 284: E946–E953, 2003. First published February 4, 2003; 10.1152/ajpendo.00397.2002.—Debilitating injury is accompanied by hypercortisolemia, muscle wasting, and disruption of the normal anabolic response to food. We sought to determine whether acute hypercortisolemia alters muscle protein metabolism following ingestion of a potent anabolic stimulus: essential amino acids (EAA). A 27-h infusion ($80\ \mu g\cdot kg^{-1}\cdot h^{-1}$) of hydrocortisone sodium succinate mimicked cortisol (C) levels accompanying severe injury ($>30\ \mu g/dl$), (C + AA; $n = 6$). The control group (AA) received intravenous saline ($n = 6$). Femoral arteriovenous blood samples and muscle biopsies were obtained during a primed ($2.0\ \mu mol/kg$) constant infusion ($0.05\ \mu mol\cdot kg^{-1}\cdot min^{-1}$) of L-[$ring$-$^2H_5$]phenylalanine before and after ingestion of 15 g of EAA. Hypercortisolemia [36.5 ± 2.1 (C + AA) vs. $9.0 \pm 1.0\ \mu g/dl$ (AA)] increased postabsorptive arterial, venous, and muscle intracellular phenylalanine concentrations. Hypercortisolemia also increased postabsorptive and post-EAA insulin concentrations. Net protein balance was blunted (40% lower) following EAA ingestion but remained positive for a greater period of time (60 vs. 180 min) in the C + AA group. Thus, although differences in protein metabolism were evident, EAA ingestion improved muscle protein anabolism during acute hypercortisolemia and may help minimize muscle loss following debilitating injury.

cortisol; stress; metabolism; injury; stable isotopes

DEBILITATING INJURY OR TRAUMA is associated with muscle wasting and a progressive loss of functional capacity. This imbalance between muscle protein synthesis and breakdown is likely facilitated by an increased circulating level of the stress hormone cortisol (20, 41). The hypercortisolemic response is generally proportional to the severity of an injury and has been shown to increase skeletal muscle proteolysis, particularly when combined with immobilization or bed rest (16, 41). The metabolic changes that result in a loss of muscle tissue following injury can be broadly characterized as occurring as a result of two main physiological responses: *1*) changes in postabsorptive protein metabolism and *2*) alteration in the anabolic response to feeding. Several studies have characterized changes in postabsorptive protein metabolism in response to hypercortisolemia (12, 16, 20, 30). In general, these studies demonstrate that, in the postabsorptive state, cortisol increases whole body proteolysis (30), increases plasma insulin levels (12, 30), and raises plasma amino acid concentrations (12, 16, 20, 30).

Although physical interventions such as exercise may provide a potent anabolic stimulus, exercise may not be a feasible countermeasure in all situations, particularly those involving injury or trauma. Consequently, there is need to examine additional strategies, such as nutrition, that may arrest the cachectic process. After severe injury or traumatic insult, the normal anabolic stimulus to feeding is disrupted. Even with tremendously elevated caloric intakes, some severely injured individuals fail to maintain lean body mass (22, 32). Furthermore, although chronic outcome measurements such as body weight, strength, and functional ability clearly demonstrate that protein catabolism exceeds muscle protein synthesis following injury, it is not clear whether this disruption of physiological homeostasis can be blunted or offset by a nutritional intervention.

We have recently shown (33, 34) in healthy subjects that ingestion or infusion of essential amino acids (EAA) provides a potent, acute anabolic stimulus. Furthermore, preliminary data from our laboratory suggest that the anabolic response to EAA ingestion is substantially greater than a mixed meal or even an intact protein source.

The purpose of this study was to determine whether acute exposure to hypercortisolemia alters muscle protein metabolism following the bolus ingestion of 15 g of EAA. To complement the protein kinetics data, we chose two independent, primary end point measurements, muscle fractional synthetic rate (FSR) and net phenylalanine balance. We hypothesized that EAA ingestion would be effective in stimulating muscle protein anabolism during acute hypercortisolemia.

METHODS

Subjects

Twelve healthy male and female subjects (mean ± SE: 28 ± 5 yr, 165 ± 6 cm, 70 ± 2 kg) were randomly assigned to one of two experimental conditions. In the amino acid group (AA; 2 males, 4 females), we used an established stable isotope methodology (9, 38) to measure muscle protein kinetics before and after a bolus ingestion of 15 g of EAA. In the second group (C + AA; 2 males, 4 females) we induced hypercortisolemia and then measured muscle protein kinetics before and after ingestion of 15 g of EAA.

Experimental Protocols

This study was approved by the Institutional Review Board at The University of Texas Medical Branch (UTMB). After the experimental protocol and risks of the study were explained, written informed consent was obtained from each volunteer. The study time line is presented in Fig. 1. At 1100 on *day 1* of the protocol, subjects were admitted to the General Clinical Research Center at UTMB, given a standard meal, and then allowed only water until the completion of the study. At 1200, an 18-gauge polyethylene catheter was inserted into a forearm vein for infusion of hydrocortisone sodium succinate (infusion rate: 80 μg·kg^{-1}·h^{-1}; Solu-Cortef, Upjohn, MI) or placebo (0.9% saline). The following morning at 0600, a second 18-gauge, polyethylene catheter was inserted into a peripheral forearm vein. After baseline blood samples were obtained, a primed (2 μmol/kg) continuous infusion (0.05 μmol·kg^{-1}·min^{-1}) of L-[*ring*-^{2}H$_5$]phenylalanine ([^{2}H$_5$]Phe) was started. At 0700, polyethylene catheters (3-Fr, 8 cm) were inserted into the femoral vein and femoral artery of one leg under local anesthesia. Arterial and venous blood samples were obtained at 10- to 20-min intervals before and after the ingestion of the EAA drink for determination of amino acid kinetics and plasma concentrations of glucose, insulin, and cortisol. The femoral artery catheter was also used for indocyanine green (ICG) infusion (infusion rate 0.5 mg/min) for the spectrophotometric (λ = 805 nm) determination of leg blood flow. Blood flow was measured on three occasions, as depicted in Fig. 1. ICG was infused into the femoral artery for ~20 min. Three blood samples (2 ml) were drawn simultaneously from the femoral and peripheral veins during *minutes 10–20* of the ICG infusion. Leg plasma flow was calculated from steady-state ICG concentrations and converted to blood leg flow by use of the hematocrit (10, 24). Values for each collection period were averaged and used to represent blood flow during *1*) the postabsorptive period, *2*) 0–120 min post-EAA drink, and *3*) 120–240 min post-EAA drink. Urine samples were collected over a 24-h period from the start of the cortisol-saline infu-

Table 1. *Distribution of essential amino acids in the 15-g EAA drink*

Amino Acid	Grams	% of Total Amino Acids
Histidine	1.64	10.9
Isoleucine	1.56	10.4
Leucine	2.79	18.6
Lysine	2.33	15.5
Methionine	0.46	3.1
Phenylalanine	2.33	15.5
Threonine	2.20	14.7
Valine	1.73	11.5
Total	15	100

EAA, essential amino acids.

sion (1200, *day 1*) for analysis of 24-h urinary cortisol levels. Muscle biopsies (50 mg) were taken from the lateral portion of the vastus lateralis ~10–15 cm above the knee with a 5-mm Bergstrom biopsy needle (4).

The composition of the EAA drink approximated the distribution of essential amino acids in skeletal muscle (Table 1). An additional 0.186 g of [^{2}H$_5$]Phe was added to the EAA drink to maintain blood isotopic enrichment. The amino acids were dissolved in 250 ml of a noncaloric, noncaffeinated soft drink and consumed as a bolus at 1100.

Analytical Methods

Blood. Femoral artery and vein blood samples were immediately mixed and precipitated in preweighed tubes containing a 15% sulfosalicylic acid solution and an internal standard. The internal standard (100 μl/ml blood) contained 49.47 μmol/l L-[*ring*-^{13}C$_6$]phenylalanine. Samples were reweighed and centrifuged, and the supernatant was removed and frozen (−80°C) until analysis. Upon thawing, 500 μl of supernatant were passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H+ form; Bio-Rad Laboratories, Richmond, VA) and dried under vacuum (Savant Instruments, Farmingdale, NY). Phenylalanine enrichments and concentrations were determined on the *tert*-butyldimethylsilyl derivative using GC-MS (HP model 5989; Hewlett-Packard, Palo Alto, CA) with electron impact ionization. Ions 336, 341, 342 were monitored (26, 36, 42). Plasma insulin and cortisol concentrations were determined by radioimmunoassay (Coat-A-Count; Diagnostic Products, Los Angeles, CA).

Muscle. Muscle biopsy samples from the vastus lateralis muscle were immediately rinsed, blotted, and frozen in liquid nitrogen until analysis. Upon thawing, samples were weighed, and the protein was precipitated with 800 μl of 14% perchloroacetic acid. To measure intracellular phenylalanine concentration, an internal standard (2 μl/mg wet wt) containing 3 μmol/l L-[*ring*-^{13}C$_6$]phenylalanine was added. Approximately 1.5 ml of supernatant were collected after tissue homogenization and centrifugation and processed in the same manner as the supernatant from blood samples (see *Blood*). Intracellular phenylalanine enrichment and concentrations were determined using the tert-butyldimethylsilyl derivative (5, 36). The remaining muscle pellet was washed and dried, and the proteins were hydrolyzed in 6 N HCl at 50°C for 24 h. The protein-bound [^{2}H$_5$]Phe enrichment was determined using GC-MS (HP model 5989, Hewlett-Packard) with electron impact ionization (13).
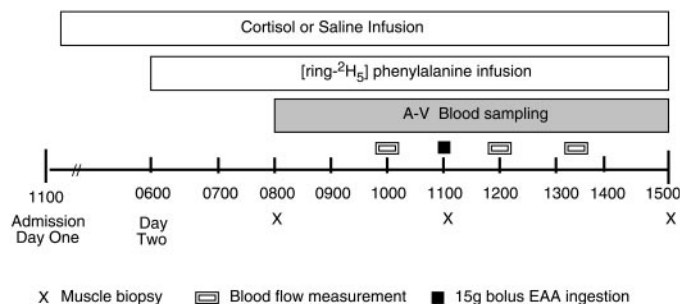

Fig. 1. Study time line and infusion protocol. A-V, arteriovenous; EAA, essential amino acids.
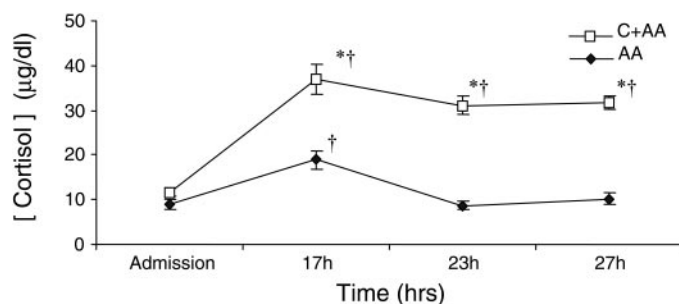
Fig. 2. Plasma cortisol levels. AA, amino acid group; C + AA, cortisol + AA group. *Significant difference from control (AA) group, $P < 0.05$. †Significant difference from baseline (pre), $P < 0.05$.

Calculations

This experimental protocol used two established methodologies to measure phenylalanine kinetics in human skeletal muscle: the two-pool arteriovenous (a-v) model and calculation of the FSR via direct phenylalanine incorporation into muscle. Phenylalanine was selected to represent amino acid kinetics because it is neither produced nor metabolized in skeletal muscle. Briefly, model parameters for phenylalanine were calculated as follows (38):

delivery of amino acids to leg: $F_{in} = C_A \cdot BF$ (1)

release of amino acids from leg: $F_{out} = C_V \cdot BF$ (2)

net muscle balance: $NB = (C_A - C_V) \cdot BF$ (3)

rate of appearance: $R_a = (E_A/E_V - 1) C_A \cdot BF$ (4)

rate of disappearance: $R_d = NB + R_a$ (5)

where C_A and C_V represent the phenylalanine concentrations in the femoral artery and vein, respectively, BF represents leg blood flow as determined by the ICG dye dilution method (24), and E_A and E_V represent the phenylalanine enrichment (tracer-to-tracee ratio) in the artery and vein. The rate of appearance (R_a) represents an estimation of the amount of phenylalanine released from breakdown that appears in the plasma, and the rate of disappearance (R_d) represents an estimation of the rate of phenylalanine incorporation of plasma phenylalanine into muscle protein. By use of average postabsorptive net balance values as a baseline, area under the curve values were used to provide an estimation of net phenylalanine uptake following EAA ingestion (31).

The FSR of mixed muscle protein was calculated by measuring the incorporation of [^{2}H$_5$]Phe into protein by use of the precursor-product model

$$FSR = [(E_{P2} - E_{P1})/(E_M \cdot t)] \cdot 60 \cdot 100   (6)$$

where E_{P1} and E_{P2} are the enrichments of bound [^{2}H$_5$]Phe in two sequential muscle biopsies, t is the time interval between biopsies, and E_M is the [^{2}H$_5$]Phe enrichment in the muscle intracellular pool (3). Postabsorptive FSR values were calculated using the average E_M values from the first two biopsies. E_M values from the final biopsy were used to calculate post-EAA ingestion FSR, as it represented the most conservative estimate of FSR during this period (see RESULTS: *Phenylalanine Enrichment and Concentration*).

Statistical Analysis

Data are presented as means ± SE. Because of unequal variances between the postabsorptive and post-EAA periods, between-group comparisons in each phase were performed

independently. t-Tests were used to compare postabsorptive data in the AA and C + AA groups. After bolus ingestion of 15 g of EAA, comparisons were performed using ANOVA with repeated measures. Differences were considered significant at $P < 0.05$.

RESULTS

Plasma and Urinary Cortisol

Plasma cortisol levels were significantly elevated in the C + AA group ($P < 0.05$; Fig. 2). Twenty-four-hour urinary cortisol levels were 67 ± 15 and 773 ± 136 µg/24 h for the AA and C + AA groups, respectively ($P < 0.05$).

Phenylalanine Enrichment and Concentration

Plasma [^{2}H$_5$]Phe enrichment in the femoral artery and vein remained at near steady state for the duration of the study. Arterial enrichments were 7.7 ± 0.4% (AA) and 7.3 ± 0.3% (C + AA; $P > 0.05$), and venous enrichments were 6.9 ± 0.4% (AA) and 6.6 ± 0.3% (C + AA), respectively ($P > 0.05$).

Arterial and venous phenylalanine concentrations are presented in Fig. 3. In the postabsorptive state, hypercortisolemia increased plasma phenylalanine concentrations. Postabsorptive arterial values were 58.8 ± 3.1 (AA) vs. 72.5 ± 4.1 nmol Phe/ml (C + AA; $P < 0.05$; Table 2). Similarly, postabsorptive venous concentrations were 64.0 ± 3.8 (AA) vs. 78.3 ± 4.0 nmol Phe/ml (C + AA), respectively ($P < 0.05$). After ingestion of 15 g of EAA, arterial and venous phenylalanine concentrations in the AA and C + AA groups increased significantly ($P < 0.05$). However, the magnitude of the arterial and venous concentration change
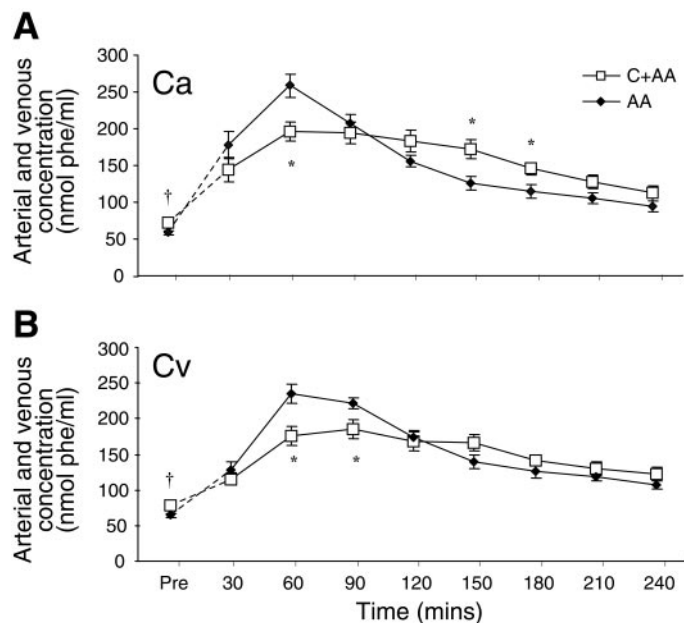

Fig. 3. Femoral artery (Ca) and vein (Cv) phenylalanine concentrations before (Pre) and after ingestion of 15 g of EAA. *Significant difference from control (AA) group, $P < 0.05$. †All post-EAA values were different from baseline (pre), $P < 0.05$.

Table 2. *Postabsorptive phenylalanine kinetics across the leg with (C + AA)*
and without (AA) hypercortisolemia

Postabsorptive Variable	Saline (AA)	Cortisol (C + AA)	P Value
Plasma cortisol, μg/dl	9.0 ± 1.0	36.5 ± 2.1	<0.001
Plasma insulin, μIU/ml	6.1 ± 1.5	11.1 ± 1.7	<0.05
Blood glucose, mg/dl	82.0 ± 2.4	109.5 ± 2.6	<0.001
Arterial Concn, nmol Phe/ml	58.8 ± 3.1	72.5 ± 3.4	0.001
Venous Concn, nmol Phe/ml	64.0 ± 3.8	78.3 ± 4.0	0.002
Muscle IC Concn, nmol Phe/ml	70.8 ± 6.7	92.5 ± 5.2	0.001
F_{in}, nmol Phe·min^{-1}·100 ml leg^{-1}	174.7 ± 18.1	289.6 ± 32.5	0.005
F_{out}, nmol Phe·min^{-1}·100 ml leg^{-1}	190.3 ± 19.7	312.2 ± 35.1	0.005
Fractional synthetic rate, %/h	0.083 ± 0.001	0.073 ± 0.007	NS
Rate of appearance, nmol Phe·min^{-1}·100 ml leg^{-1}	36.5 ± 5.7	45.5 ± 4.8	NS
Rate of disappearance, nmol Phe·min^{-1}·100 ml leg^{-1}	20.9 ± 4.4	22.9 ± 3.1	NS
Net balance, nmol Phe·min^{-1}·100 ml leg^{-1}	-15.6 ± 1.9	-22.6 ± 7.5	NS
Leg blood flow (ml·min^{-1}·100 ml leg^{-1}	3.0 ± 0.3	4.0 ± 0.4	NS

Values are means $\pm$ SE from the postabsorptive period. AA, amino acid group; C + AA, cortisol + AA group; IC, intracellular; F_{in}, delivery of AA to the leg; F_{out}, release of AA from the leg; NS, not significant.

was significantly blunted by cortisol infusion [arterial peak values: 272.4 ± 12.1 (AA) vs. 208.1 ± 17.8 nmol Phe/ml (C + AA), $P < 0.05$]. Moreover, hypercortisolemia resulted in arterial phenylalanine concentrations remaining elevated for a longer period of time after EAA ingestion (Fig. 3).

Muscle enrichment increased from $4.6 \pm 0.5\%$ (AA) and $4.9 \pm 0.4\%$ (C + AA) during the postabsorptive period to $6.2 \pm 0.5\%$ (AA) and $6.4 \pm 0.3\%$ (C + AA; $P < 0.05$) at the time of the final biopsy (4 h after EAA ingestion). This increase in muscle enrichment was likely facilitated by an increase in the intracellular pool size caused by an increased inward transport of phenylalanine with no concomitant change in breakdown.

During the postabsorptive period, hypercortisolemia increased muscle intracellular phenylalanine concentration with values of 70.8 ± 6.7 (AA) vs. 92.5 ± 5.2 nmol/ml (C+AA; $P < 0.05$). EAA ingestion further increased muscle intracellular phenylalanine concentrations, with the greater increase occurring in the C + AA group. Post-EAA values (*biopsy 3*) were 115.6 ± 5.4 (AA) vs. 178.8 ± 18.6 nmol/ml (C + AA), respectively ($P < 0.05$; Fig. 4).
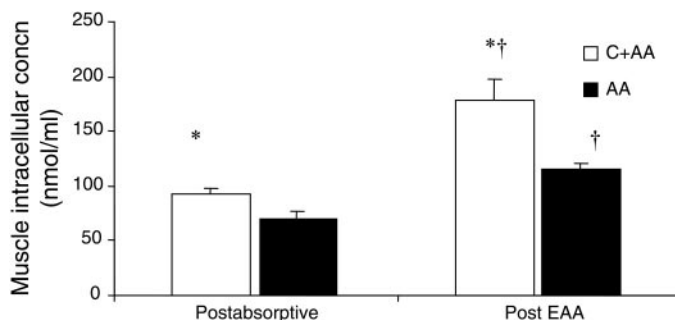

Fig. 4. Muscle intracellular phenylalanine concentrations from biopsy samples obtained during the postabsorptive period and 4 h after EAA ingestion. *Significant difference from control (AA) group, $P < 0.05$. †Significant difference from the postabsorptive period, $P < 0.05$.

Blood Flow

EAA ingestion did not alter leg blood flow ($P > 0.05$). Blood flow values were consistently higher in the C + AA group but did not reach statistical significance. AA blood flow values were 3.0 ± 0.3 (postabsorptive), 3.2 ± 0.3 (0–120 min post-EAA), and 3.2 ± 0.4 ml·min^{-1}·100 ml leg vol^{-1} (120–240 min post-EAA). C + AA blood flow values were 4.0 ± 0.4 (postabsorptive), 3.82 ± 0.4 (0–120 min post-EAA), and 3.9 ± 0.3 ml·min^{-1}·100 ml leg vol^{-1} (120–240 min post-EAA; $P > 0.05$).

Amino Acid Delivery to and Release from the Leg

In the postabsorptive state, hypercortisolemia resulted in a greater flow of amino acids into and out of the leg ($P < 0.05$; Table 2). Amino acid delivery to (F_{in}) and release from (F_{out}) the leg increased significantly following EAA ingestion. Hypercortisolemia significantly prolonged the elevation of both variables ($P < 0.05$; Fig. 5).

R_a and R_d

During the postabsorptive period, phenylalanine R_a, a reflection of leg muscle protein breakdown, was not significantly increased by acute hypercortisolemia, with values of 36.5 ± 5.7 (AA) and 45.5 ± 4.8 nmol Phe·min^{-1}·100 ml leg vol^{-1} (C + AA), respectively ($P > 0.05$). After EAA ingestion, R_a values remained relatively stable, with period average values of 40.7 ± 8.4 (AA) and 40.1 ± 8.3 nmol Phe·min^{-1}·100 ml leg vol^{-1} (C + AA), respectively ($P > 0.05$).

During the postabsorptive period, hypercortisolemia did not alter phenylalanine R_d, a reflection of muscle protein synthesis. Values were 20.9 ± 4.4 (AA) and 22.9 ± 3.1 nmol Phe·min^{-1}·100 ml leg vol^{-1} (C + AA), respectively ($P > 0.05$). After EAA ingestion, R_d was blunted by hypercortisolemia yet remained above postabsorptive levels for a greater period of time. R_d increased in both groups 30 min after EAA ingestion ($P < 0.05$) but was significantly greater in the AA group,
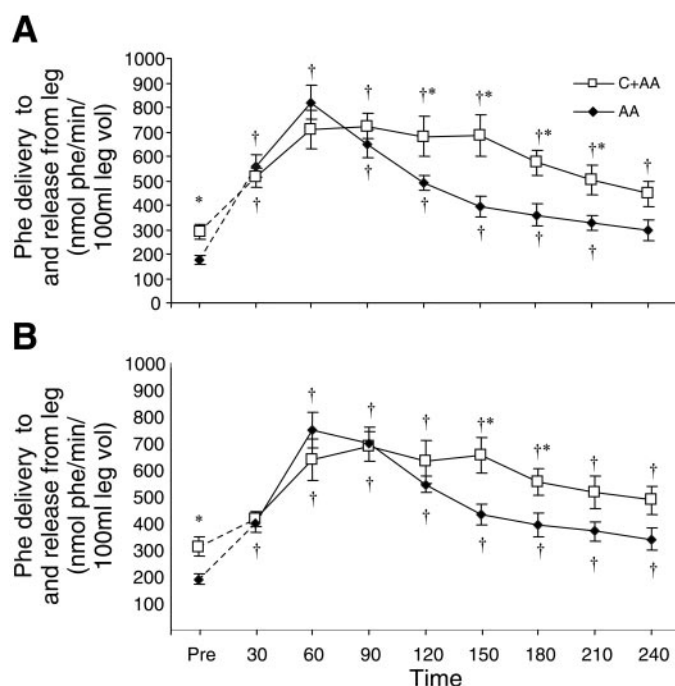
Fig. 5. Amino acid delivery (*A*; F_{in}) to the leg and release (*B*; F_{out}) from the leg before (Pre) and after ingestion of 15 g of EAA. *Significant difference from control (AA) group, $P < 0.05$. †Significant difference from baseline (pre), $P < 0.05$.
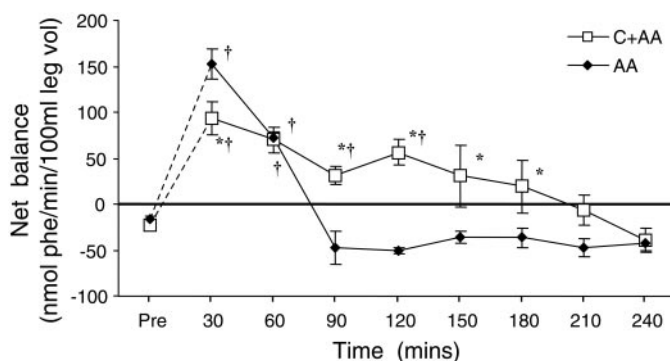

Fig. 6. Phenylalanine net balance across the leg before (Pre) and after ingestion of 15 g of EAA. *Significant difference from control (AA) group, $P < 0.05$. †Significant difference from baseline (pre), $P < 0.05$.

with values of 204.4 ± 18.2 (AA) and 133.8 ± 18.3 nmol Phe·min^{-1}·100 ml leg vol^{-1} (C + AA), respectively ($P < 0.05$). By 90 min, R_d was not different from postabsorptive levels in the AA group ($P > 0.05$). In the C + AA group, R_d was still 75% higher than postabsorptive levels at 120 min after EAA ingestion and remained significantly higher than the AA group values for 210 min following EAA ingestion ($P < 0.05$).

Net Balance

No differences in net phenylalanine balance (NB) were identified while subjects remained in the postabsorptive state, with values of -15.6 ± 1.9 (AA) and -22.6 ± 7.5 nmol Phe·min^{-1}·100 ml leg vol^{-1} (C + AA), respectively ($P > 0.05$). On the basis of power calculations ($\alpha = 0.05$, $\beta = 80\%$), we would have needed to study 23 subjects in each group to reach statistical significance. Both groups experienced a significant increase in NB following ingestion of 15 g of EAA; however, the response in the C + AA group was blunted but remained positive for a greater period of time (Fig. 6). Calculation of net phenylalanine uptake (NB area under the curve: above baseline NB = 0) by the leg for the postdrink periods also reflects the blunted, yet prolonged period of positive NB associated with hypercortisolemia. Net phenylalanine uptake during the 1-h postdrink period was 102.0 ± 6.0 (AA) vs. 77.9 ± 8.3 mg Phe/leg (C + AA; $P < 0.05$). Values were similar 90 min after EAA ingestion, 101.8 ± 8.2 (AA) vs. 104.3 ± 9.9 mg Phe/leg (C + AA; $P > 0.05$), whereas net phenylalanine uptake over the 4-h post-

drink period was 36.0 ± 19.2 (AA) vs. 167.1 ± 29.8 mg Phe/leg (C + AA; $P < 0.05$) (Fig. 7).

Muscle FSR

Both groups experienced an increase in mixed muscle FSR in response to ingestion of 15 g of EAA ($P < 0.05$). However, no between-group differences were evident during the pre- or postdrink periods (AA: 0.083 ± 0.010 and $0.109 \pm 0.011\%$/h; C + AA: 0.073 ± 0.007 and $0.111 \pm 0.018\%$/h; $P > 0.05$). Similarly, hypercortisolemia did not significantly affect absolute change in FSR following EAA ingestion, with increases of 0.027 ± 0.013 (AA) and $0.038 \pm 0.019\%$/h (C + AA), respectively.

Insulin and Glucose

Postabsorptive plasma insulin concentrations were elevated in response to hypercortisolemia, with values of 6.1 ± 1.5 (AA) and 11.1 ± 1.7 µIU/ml (C + AA), respectively ($P < 0.05$). After EAA ingestion, insulin levels increased in both groups ($P < 0.05$) but remained significantly higher in the C + AA group ($P < 0.05$; Fig. 8).

Hypercortisolemia also increased plasma glucose concentrations, with values of 82.0 ± 1.5 (AA) and 109.4 ± 3.5 mg/dl (C + AA), respectively ($P < 0.05$).
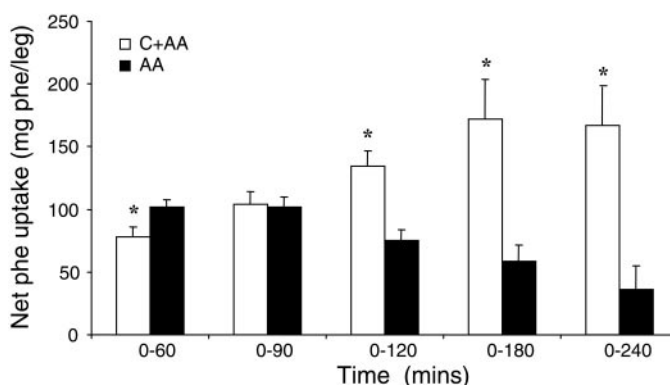

Fig. 7. Net phenylalanine uptake by the leg at 0–60, 0–90, 0–120, 0–180, and 0–240 min after 15 g of EAA ingestion. *Significant difference from control (AA) group, $P < 0.05$.
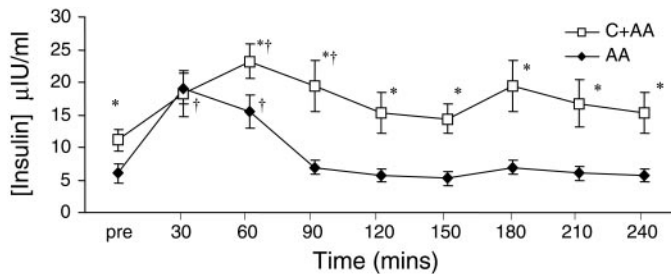
Fig. 8. Plasma insulin concentrations before (Pre) and after ingestion of 15 g of EAA. *Significant difference from control (AA) group, $P < 0.05$. †Significant difference from baseline (pre), $P < 0.05$.

DISCUSSION

After severe injury, individuals continue to lose muscle mass despite elevated caloric intake (22, 32). This loss of lean body mass, although partly the result of reduced physical activity, is likely facilitated by a concomitant increase in plasma cortisol levels (20, 41). Normal circulating levels of cortisol follow a diurnal pattern but are typically in the range of 5–15 μg/dl. The goal of this study was to induce acute hypercortisolemia ($\sim$35 μg/dl) to mimic levels observed after a traumatic event and then examine muscle phenylalanine kinetics in response to a known potent anabolic stimulus, EAA ingestion. We found that, although muscle phenylalanine kinetics were altered by acute hypercortisolemia, ingested EAA were still able to stimulate muscle protein synthesis and acutely reverse negative net phenylalanine balance.

Our data were consistent with a previous study (21), which demonstrated that a 6-h infusion of mixed amino acids, given in conjunction with a stress hormone infusion, was able to maintain skeletal muscle ribosome distribution and size, a representative marker of protein synthesis. The present study is the first to investigate the time course and progression of the interaction between acute hypercortisolemia and protein metabolism in response to a bolus oral ingestion of an EAA solution.

Several investigators have described an increase in proteolysis in hypercortisolemic ($>$30 μg/dl) postabsorptive subjects (12, 15, 20, 30). These studies estimated whole body protein breakdown by use of a methodology based on arterialized peripheral venous blood samples. In the present study, we employed the leg a-v model to characterize muscle phenylalanine kinetics in a single limb and found that hypercortisolemia increased postabsorptive phenylalanine concentrations in the vastus lateralis and femoral artery and vein but did not alter postabsorptive net phenylalanine balance or muscle FSR. Furthermore, despite an upward trend, hypercortisolemia did not result in a significant increase in phenylalanine R_a, an indirect indicator of muscle protein breakdown in the leg. Although methodological differences between studies using the leg two-pool (a-v) (16) and whole body models (12, 15, 20, 30) may have contributed to the failure to detect a significant increase in postabsorptive protein break-

down (R_a) with hypercortisolemia, the relatively short duration of hypercortisolemic period and/or the methodology may not have provided sufficient sensitivity to detect small changes in some variables. Alternatively, these data may simply reflect the very early stages of the catabolic process. Indeed, the increased circulating and muscle intracellular phenylalanine concentrations noted during the postabsorptive period raise the possibility that acute exposure to elevated cortisol levels primes the system by raising energy expenditure, increasing amino acid turnover, and increasing amino acid movement between pools (12). The net loss of nitrogen from muscle may not actually occur unless hypercortisolemia is prolonged, combined with inactivity (16), and/or combined with other mediators of the injury/trauma response (6, 41).

Ingestion or infusion of EAA provides a strong anabolic stimulus (33, 34, 39). Consistent with these previous data, we observed a significant increase in mixed muscle FSR in both experimental conditions during the 4-h period following EAA ingestion. However, although hypercortisolemia increased muscle intracellular phenylalanine concentrations, it did not affect FSR during either the postabsorptive or the post-EAA periods. Nevertheless, by use of the net balance approach to estimate the relationship between protein synthesis and breakdown, it is clear that hypercortisolemia acutely and transiently altered phenylalanine kinetics immediately following EAA ingestion. The blunted, yet prolonged period of positive net balance was facilitated by an increase in femoral a-v amino acid concentrations, slightly higher blood flow values, and a resultant greater delivery of amino acids to the leg. These concentration differences observed during hypercortisolemia were also associated with a greater net phenylalanine uptake and a blunted, yet prolonged post-EAA increase in the phenylalanine R_d, an indirect measure of protein synthesis.

The mechanisms resulting in the altered phenylalanine concentrations and a-v model kinetics during hypercortisolemia are not entirely clear. It is possible that a slower rate of gastric emptying or impaired absorption may have contributed to the blunted, yet prolonged increase in plasma phenylalanine concentration in the C + AA group. However, there is no clear evidence that hypercortisolemia alters amino acid digestion or absorption. Phenylalanine is an essential amino acid and is not synthesized or metabolized in the body; consequently, changes in the R_a or R_d of phenylalanine in the plasma reflect protein synthesis and breakdown (37). However, the prolonged elevation in net phenylalanine balance and R_d may not necessarily reflect a similar time course of increased protein synthesis. In a recent study (11), multiple muscle biopsies were taken from the vastus lateralis during a period when plasma amino acid concentrations were elevated via constant intravenous infusion. Although muscle protein synthesis (FSR) was acutely stimulated by the increased amino acid availability, FSR returned to basal levels after $\sim$2 h despite the elevated plasma concentrations (11). In the present study, plasma

amino acid concentrations remained elevated for 4 h after EAA ingestion. However, if the majority of protein synthesis occurred during the 2-h period following EAA ingestion (11), it may account for the similarities in the FSR values in both groups and would provide at least a partial explanation for the greater net phenylalanine uptake observed between 2 and 4 h following EAA ingestion during hypercortisolemia (Fig. 7).

The fact that there was an increase in arterial and venous phenylalanine concentrations, yet only a trend toward an increase in muscle protein breakdown (R_a) during hypercortisolemia, suggests that increased protein breakdown in nonmuscle tissues may have contributed to the elevated plasma amino acid levels during the pre- and post-EAA drink periods. The source of this potential increase in nonmuscle proteolysis is unclear. Certainly, the liver represents a large potential source of amino acids that could be rapidly released into the circulation given an appropriate signal. However, although there are no definitive data on overall net protein balance in the liver during hypercortisolemia, a number of reports suggest that glucocorticoids stimulate uptake of amino acids by the liver and promote acute-phase protein synthesis (17, 27).

There are some data suggesting that the gut may provide a source of amino acids during periods of exercise-induced stress (23, 35). Using a dog model with an exercise intervention, Williams et al. (35) reported an increased release of leucine from the splanchnic bed, with the primary contributing site being the gut. Certainly, given the high protein turnover rate in the gut, even a slight imbalance between synthesis and breakdown could produce relatively large changes in plasma amino acid concentrations.

Although the site of increased proteolysis cannot be addressed directly by this study, the prolonged increase in net phenylalanine balance and the greater net phenylalanine uptake in the C + AA group are perhaps best explained as the result of plasma concentration changes facilitating a more sustained delivery of amino acids to the leg (F_{in}). Whether these changes are the direct result of elevated cortisol levels or the interaction of several secondary homeostatic changes remains uncertain.

The infusion of cortisol to produce hypercortisolemia in healthy human subjects has broad-ranging effects and ultimately influences a range of hormonal responses including the regulation of glucose metabolism. Consequently, it is possible that the altered phenylalanine kinetics observed during hypercortisolemia may also have been influenced by the concomitantly elevated plasma insulin levels both before and after ingestion of 15 g of EAA. The mechanisms by which insulin regulates protein metabolism are complex, and different organ systems and tissues may be affected in a variable manner [for a detailed review see Wolfe and Volpi (40)]. Although there remains a degree of ambiguity regarding the role of insulin on the in vivo regulation of muscle protein synthesis and breakdown (2, 18, 40), insulin has been shown to blunt the proteolytic effects of hormonally induced catabolism (6, 7, 14).

Previously, researchers have used somatostatin to modulate insulin release to partially differentiate between the effects of hypercortisolemia and hyperinsulinemia (12). However, somatostatin has several potentially confounding effects on gastrointestinal function, including *1*) inhibited gastric secretions, *2*) reduced splanchnic blood flow, *3*) inhibited intestinal absorption, and *4*) reduced gastrointestinal motility (28), which makes it unsuitable for use in association with oral EAA ingestion.

In the present study, we found that hypercortisolemia increased plasma glucose concentrations despite an accompanying increase in insulin levels (12, 15, 19, 30). This elevation in plasma glucose levels was most likely due to a reduction in the rate of glucose disposal and not an increase in production (12, 25, 29). The suggestion that there was no increased requirement for glucose is congruent with the theory that acute exposure to hypercortisolemia primes the muscle for the subsequent use of glucose and amino acids for energy production if an additional stimulus presents (i.e., prolonged hypercortisolemia, inactivity, physical trauma).

In conclusion, the pattern of the anabolic response to amino acid ingestion is altered by acute hypercortisolemia. Nevertheless, dietary amino acids are still able to effectively stimulate muscle protein synthesis and reverse the negative net phenylalanine balance associated with hypercortisolemia. Although EAA ingestion may provide an effective means of promoting muscle protein anabolism during the first few days following debilitating injury, it remains to be seen whether EAA supplementation can ameliorate muscle protein loss in individuals experiencing chronic hypercortisolemia as a result of trauma.

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